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Dynamic shaping of the defensive peripersonal space through predictive motor mechanisms: when the "near" becomes "far"

Running title: Hand blink reflex and motor prediction

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Abstract

The Hand Blink Reflex (HBR) is a subcortical defensive response, known to dramatically increase when the stimulated hand is statically positioned inside the defensive peripersonal space (DPPS) of the face. Here, we tested in a group of healthy human subjects the HBR in dynamic conditions, investigating whether the direction of the hand movements (up-to/down-from the face) could modulate it. We found that, on equal hand position, the response enhancement was present only when the hand approached to (and not receded from) the DPPS of the face. This means that, when the hand is close to the face but the subject is planning to move the hand down, the predictive motor system can anticipate the consequence of the movement: the “near” becomes “far”. We found similar results both in passive movement condition, when only afferent (visual and proprioceptive) information can be used to estimate the final state of the system, and in motor imagery task, when only efferent (intentional) information are available to predict the consequences of the movement. All these findings provide evidence that the DPPS is dynamically shaped by predictive mechanisms run by the motor system and based on the integration of predictive feedforward and sensory feedback signals.

Significance Statement

The defensive peripersonal space (DPPS) has a crucial role for survival and its modulation is fundamental when we interact with the environment, as when we move our arms. Here, we focused on a defensive response, the Hand Blink Reflex (HBR), known to dramatically increase when a static hand is stimulated inside the DPPS of the face. We tested the HBR in dynamic conditions (voluntary, passive and imagined movements) and we found that, on equal hand position, the response enhancement was present only when the hand approached to (and not receded from) the DPPS of the face. This suggests that, through the integration of efferent and afferent signals, the safety boundary around the body is continuously shaped by the predictive motor system.

Introduction

The peripersonal space (PPS) is the space directly surrounding the body (Rizzolatti et al., 1997) within we can act and interact. According to a recent review (de Vignemont and Iannetti, 2015), there is not a single representation of PPS but a dual model of peripersonal space. This is based on a functional distinction between goal-directed action and bodily protection. In the present study we focused on the latter concept, that is the defensive peripersonal space (D)PPS (Cooke and Graziano, 2003; Graziano and Cooke, 2006). The DPPS has been recently investigated in humans by recording the Hand Blink Reflex (HBR), which is a subcortical response at the brainstem level elicited by the electrical stimulation of the median nerve at the wrist and recorded from the orbicularis oculi muscles (Sambo et al., 2012a, 2012b; Sambo and Iannetti, 2013; Fossataro et al., 2016). In static condition, the HBR is modulated by the hand position in space: the response dramatically increases when the stimulated hand is located close to the face, inside the DPPS.

When we interact with the surrounding environment the modulation of the DPPS can become fundamental to prevent potentially dangerous situations. During voluntary movements, the central nervous system can estimate the final hand position using either motor outflow or sensory inflow (i.e., visual and proprioceptive inputs). These two sources of information can be combined in a predictive model, according to which, once the motor program is selected and sent to the periphery, an efference copy is formed to predict the future body state and the consequences of the movement, that, in turn, are compared with the actual state detected from the sensory feedback (Wolpert et al., 1995; Blakemore et al., 2002; Haggard, 2005).

Understanding the role of the predictive motor system in modulating the DPPS during movement might be a first important step toward a full comprehension of the defensive mechanisms in ecological contexts when humans move in a possible dangerous environment. To this aim, we investigated the role of predictive motor mechanisms in dynamically shaping the DPPS during upper limb voluntary movements. To this goal, we recorded HBR when participants were asked to move their right forearm

up towards the face (up-moving condition) or down far from the face (down-moving condition). Indeed, movements in different directions could allow us to investigate the response to a dangerous stimulus entering or leaving our DPPS. In each condition, the HBR was elicited during the forearm's movement when the amplitude of the elbow angle reached three pre-defined values. In turn, these three positions determined three hand distances with respect to the face (far, intermediate and near).

We hypothesized that the predicted final consequence of the movement (either close to or far from the face) could affect the reflex response amplitude. Thus, on equal hand positions, comparing the up-moving with the down-moving condition, we expected to find a different modulation of the HBR depending on the direction of the hand movement.

During voluntary movements, intentional outflow and sensory inflow are both available to estimate the final position of the hand. Thus, to investigate the relative roles of these complementary sources of information in dynamically modulating HBR amplitude during movement, we designed two experiments, employing either passive movements (where only sensory inflow is present) or motor imagery (where, on the opposite, only intentional outflow is present). In the former experiment, the subjects were asked to stay relaxed while the examiner passively moved their right arm up towards or down far from their face; in the latter, the subjects stayed still, keeping their right hand in the intermediate position while imaging to move it up-to or down-from the face.

Materials and Methods

Participants

Thirteen participants, naive to the purpose of the experiment, were recruited for this study. They reported no previous history of neurological disorders or orthopedic problems for the right-dominant hand, as determined by the Edinburgh Handedness Inventory (Oldfield, 1971).

Firstly, participants were tested to assess whether they showed a reproducible HBR (Sambo et al., 2012a) (see Preliminary experiment). Ten of them (about the 77% of the total number, 6 females and 4 males, mean age±std = 22.4±2.3) satisfied this requirement and were thus chosen to advance to the next stages of the experimental procedure. Participants gave written informed consent before taking part in the study. The study has been approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

Experimental set up

The HBR response was elicited by administering transcutaneous electrical stimuli to the median nerve at the right wrist, using a surface bipolar electrode attached with a velcro strap and connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). As the stimulator provided constant current pulses, the trial-to-trial variability of the intensity of stimulation was negligible. Stimulus intensity was adjusted to elicit in each participant clear HBR responses (mean stimulus intensities were 26.2±4.6 mA, range 15-30 mA). None of the participants reported painful sensations elicited by the stimulation. The stimulus duration was 200 μs and the inter-stimulus interval was ~30 s. A twin-axis electronic goniometer (TSD130B, BIOPAC System, Inc.) connected to a BIOPAC MP100 system was used to measure and record the elbow angle during movement execution. In Experiment 1 and in Experiment 2 this device allowed the automatic delivery of the electrical stimulation when the elbow angle corresponded to one of the three pre-determined stimulation positions.

EMG activity was recorded by means of two MP100 BIOPAC EMG channels from the orbicularis oculi muscle bilaterally, using two pairs of bipolar surface electrodes with the active electrode over the mid lower eyelid and the reference electrode laterally to the outer canthus. Signals were amplified and digitized at 1 kHz (BIOPAC MP100).

Experimental Procedure

The experiments took place in four different sessions. In the first session participants performed the Preliminary experiment during which the HBR responses were acquired in Static condition. Participants who showed a reproducible HBR in Static condition advanced to the next stages of the study. In the second session the selected participants executed Experiment 1 (Voluntary movement). Experiment 2 (Passive movement) and the Experiment 3 (Motor imagery) were randomly executed in two other different sessions. At least one week passed between one experimental session and the following.

Participants were seated on a comfortable chair and kept the right elbow at the limit of a table, in a position allowing the right wrist to be in front of the ipsilateral eye while moving the forearm towards the face, but never touching it. The electrical stimulation was delivered, in static condition or during voluntary and passive movements, while participant's stimulated hand was located at three different positions relative to the face. In particular, when the elbow angle was 10° less than the maximal arm extension (far position, $\alpha 1$), the half of the difference between the angles of maximal arm extension and flexion (intermediate position, $\alpha 2$), and when the angle was 10° more than the maximal elbow flexion (near position, $\alpha 3$). Throughout the experiment participants were instructed to keep their gaze on a fixation point placed at 60 cm from the eyes.

Preliminary experiment: Static condition. This experiment aimed to make an initial selection of those participants who showed a reproducible HBR response. These subjects were admitted to the next sessions of the study. Further, with this study we would like to test the reliability of our set up by replicating the results known in literature. Participants were instructed, trial by trial, to put the arm in one of the three positions previously identified. After a randomly variable delay, the subject received the electrical stimulation, which was manually delivered by the experimenter. Twenty-four

157 acquisitions were performed, 8 for each hand position. The order of the hand positions at which the
158 participant received the electrical stimulus was pseudo-random.

159

160 Experiment 1: Voluntary movement (Figure 1A). The aim of the present experiment was to assess
161 whether the HBR response was modulated during voluntary movement execution and was influenced
162 by movement direction. Participants were asked to perform two sequences of movements with the
163 right arm: elbow flexion-extension (block A) and elbow extension-flexion (block B). These two
164 blocks were introduced to avoid that participants could predict the instant of the stimulation, and the
165 order of blocks execution was balanced across participants. In both blocks, the electrical stimulation
166 was delivered in each trial (flexion-extension or extension-flexion movements) during either elbow
167 flexion (afterwards called Up-moving condition) or elbow extension (afterwards called Down-
168 moving condition), when the angle measured by the goniometer reached one of the pre-set angle
169 values (α1, α2, α3). At this time, an electrical signal was automatically generated by the goniometer
170 and triggered the onset of the electrical stimulation. Ninety-six trials (2 blocks, 3 angles, 2 movement
171 directions and 8 repetitions) were acquired. A minimum time of 30 sec was kept as inter-trial interval.
172 During this interval the subjects were asked to keep the arm relaxed. The Voluntary movement
173 condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x
174 3 stimulation positions before and after voluntary movement, for a total of 24 trials). This latter
175 condition was introduced here and in each of the following experiments to test whether subjects' HBR
176 responses in the three stimulation positions were comparable in the different days. Furthermore, this
177 evaluation allowed testing possible effects on HBR amplitude due to habituation.

178

179 Experiment 2: Passive movement (Figure 1B). This experiment was performed to test the role that
180 afferent (i.e., visual and proprioceptive signals) inputs could play in modulating the HBR response.
181 Participants were asked to keep the right arm completely relaxed in a plastic splint while the

experimenter moved it by means of a transparent wire connected to the splint and a pulley system. The passive movement was an elbow flexion-extension of the right arm. A second transparent wire was attached to the opposite site of the hand support (hand back) and adjusted in length to avoid that the distance between the hand and the face of the participant was less than 4 cm. At the beginning, the experimenter asked the participant to perform an elbow flexion and extension movement at natural velocity. When the experimenter thought to have understood participant's natural movement velocity, she moved participants' forearm and verbally questioned the subjects if he/she felt the movement velocity similar to his/her velocity. After participant's agreement the experiment started. No differences were found between angular movement velocities evaluated in the Experiment 1 and the Experiment 2 (mean±SD: Experiment 1, 100.13±37 deg/s and Experiment 2, 99.58±23 deg/s; p=0.95).

The electrical stimulation was delivered during the passive movement when the angle measured by the goniometer reached the pre-set angle values (α1, α2, α3) during either elbow flexion (Up-moving condition) or extension (Down-moving condition) movements. As in the Experiment 1, an electrical signal, automatically generated by the goniometer, triggered the onset of the electrical stimulation. Differently from the Experiment 1, in order to reduce participants' expectancy, we introduced catch trials. We didn't introduce the blocks paradigm used in the Experiment 1 because in that condition no significant difference was found between the two blocks (see Results section). This allowed us to dramatically reduce the number of trials. Excluding the catch trials, a total of 48 trials (3 angles, 2 directions, 8 repetitions) were acquired. The Passive movement condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after passive movement, for a total of 24 trials).

Experiment 3: Motor imagery (Figure 1C). Before starting the experimental procedure, all the participants completed the Italian version of the Movement Imagery Questionnaire (MIQ-R; (Hall

and Martin, 1997)) to assess their ability to form kinesthetic and visual images. The MIQ-R is an 8-item self-report questionnaire, in which participants rated the vividness of their mental representations using two 7-point scales (associated to visual and kinesthetic imagery): 1 means ‘‘really easy to feel/see’’ whereas 7 corresponds to ‘‘really difficult to feel/see’’. All participants considered it fairly easy to form motor images and the scores indicated that they possessed good motor imagery abilities (mean ± SD = 18.8 ± 5.55). After that, they were instructed to put the arm in α1 and α3 positions in order to memorize them. Then, they were asked to keep the right arm in the position corresponding to α2 (intermediate position) and to kinesthetically imagine the right arm making a flexion (Up-moving condition, from α2 to α3) or an extension (Down-moving condition, from α2 to α1) movement at spontaneous velocity. At the beginning, for few trials, participants had to verbally report when the upper arm reached α1 or α3. When the experimenter learnt the time used by the participant to imagine to move towards one of the two positions, the experiment started. After each trial the subject was questioned whether the electrical stimulation was administered in correspondence to the position set for the current trial (α1 or α3), and imagined by the subject. In case of mismatch, the trial was repeated. As in the Experiment 2, catch trials were introduced to reduce participants’ expectancy of the stimulus. Excluding the catch trials, a total of 16 trials were recorded for each subject (2 imagined directions, 8 repetitions). The Motor imagery condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and after motor imagery, for a total of 24 trials).

--- Figure 1 here ---

Data processing and statistical analysis

A custom made MatLab software was used to process the EMG signals. EMG signals from each participant were filtered and rectified. HBR responses were averaged separately in each condition and for each participant. Trials with an abnormal EMG activity preceding the HBR response were

discarded by the analysis. The area under the curve (AUC, mV*ms) of each HBR average waveform was considered as outcome parameter. To compute AUC in each averaged EMG trace the software automatically analyzed a 130 ms-time interval from the stimulus onset that always contained the subject's blink. The resulting curve was then integrated to compute AUC. In all experiments, data were averaged across ipsilateral and contralateral recording sides (right and left eyes) according to the previous analyses proposed in literature (Sambo et al., 2012a).

In the Preliminary experiment, AUC values acquired in Static condition were compared by mean of a repeated-measure ANOVA with POSITION (3 levels: α1, α2 and α3) as within-subject factor. This analysis was used to confirm the literature and thus assess the reliability of our experimental set up. Further, it allowed us to identify the participants who showed a reproducible HBR response.

In Experiment 1, Experiment 2 and Experiment 3 the HBR values acquired in Static condition, before and after the different "dynamic" conditions, were subjected to three repeated-measure ANOVA (RM-ANOVA) with POSITION (3 levels: α1, α2 and α3) and TIME (2 levels: pre, post), as within-subject factors.

Furthermore, in order to compare the AUC values associated to the Static condition in Experiment 1, 2 and 3 with those of the Preliminary experiment, the AUC values in the pre and post conditions of Experiment 1, 2 and 3 were averaged. Then, these data were statistically compared by means of a RM-ANOVA with POSITION (3 levels: α1, α2 and α3) and SESSION (4 levels: Preliminary experiment, Experiment 1, Experiment 2 and Experiment 3) as within-subject factors.

In the Experiment 1, AUC data were analyzed by mean of RM-ANOVA, with POSITION (3 levels: α1, α2 and α3), BLOCK (2 levels: A and B), and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving), as within-subject factors. Furthermore, the HBR responses during voluntary movements (AUC values averaged over the blocks) were compared to those acquired in the corresponding Static condition (data were obtained by averaging AUC values evaluated in the pre and post conditions) by means of a RM-ANOVA with CONDITION (3 levels: Static, Up-moving,

Down-moving) and POSITION (3 levels: α1, α2 and α3), as within-subject factors. In the Experiment 2, AUC values were statistically analyzed by mean of a RM-ANOVA with POSITION (3 levels: α1, α2 and α3) and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving). In order to compare AUC values evaluated during passive movement with those in Static condition (data were obtained by averaging AUC values evaluated in the pre and post conditions), a RM-ANOVA with CONDITION (3 levels: Static, Up-moving, Down-moving) and POSITION (3 levels: α1, α2 and α3), as within-subject factors, was performed. In the Experiment 3, a paired t-test (2 levels: Up-moving, Down-moving), was adopted to evaluate HBR responses during the imagination of flexion and extension movements. Further, we performed an additional analysis where a baseline condition, during which the HBR response in Static condition corresponding to α2, was directly compared to HBR amplitudes during MI in both up-moving and down-moving conditions (RM-ANOVA, within factor CONDITION, 3 levels: Static α2, MI Up-moving, MI Down-moving). This allowed us to go deeper insight the mechanisms regulating the reflex response when the arm is actually in a Static condition but the motor system is involved in movement planning. Newmann-Keuls post hoc analysis was used to interpret significant interactions. Data in the text are reported as mean ± SE.

Results

Preliminary experiment: Static condition (Figure 2). The statistical analysis showed a significant effect of the factor POSITION ($F(2,18)=7.49$, $p=0.004$). Post hoc tests revealed a significant increase of AUC values in α3 (22.33±2.55 mV*ms) with respect to α1 (17.86±2.32 mV*ms, $p=0.02$) and α2 (15.06±1.26 mV*ms, $p=0.003$). These results confirmed the literature showing that when the stimulated arm is close to the face, inside the DPPS, the HBR magnitude is significantly higher than those evoked when the arm is in farther positions.

--- Figure 2 here ---

Comparison among the Static conditions acquired in the four testing sessions. The results of the RM-ANOVA on AUC values related to the Static conditions acquired in each testing session showed a significant effect of POSITION ($F(2,18)=17.87$, $p=0.00005$), due to the significant increase of α3 with respect to the other stimulation positions (p always < 0.004). No differences appeared among the SESSIONS ($p=0.33$).

Experiment 1: Voluntary movement. Single-subjects averaged data in each conditions are shown in Figure 3. RM-ANOVA analysis showed that MOVEMENT DIRECTION ($F(1,9)=5.66$, $p=0.04$) as well as POSITION ($F(2,18)=6.94$, $p=0.006$) significantly affected the amplitude of the HBR responses and a significant interaction between these two factors was found ($F(2,18)=8.34$, $p=0.003$). Post hoc analysis showed that during an elbow flexion movement (Up-moving condition) there was a significant increase of the HBR value in α3 (12.3±1.7 mV*ms) with respect to α2 (8±1.1 mV*ms, $p=0.0003$) and α1 (8.7±1 mV*ms, $p=0.001$) (Figure 4A). Differently, during elbow extension movements (Down-moving condition) no difference in the AUC values was found in the three stimulation positions (α1=9.3±1.3 mV*ms, α2=8.9±1.1 mV*ms, α3=9.3±1.1 mV*ms, p always > 0.7) (Figure 4B). Further, the HBR response in α3 when moving up to the face was significantly higher than that observed at the same position when the hand moved far from the face ($p=0.001$). Finally, no difference between the two experimental blocks (i.e., elbow flexion-extension and elbow extension-flexion) was found ($p=0.23$).

RM-ANOVA on AUC values evaluated in the Static condition revealed an effect of the factor POSITION ($F(2,18)=11.21$, $p=0.0006$) due to the significant increase of the AUC values in α3 (25.24±3.34 mV*ms) with respect to α2 (18.15±3.3 mV*ms, $p=0.0009$) and α1 (19.56±3 mV*ms, $p=0.002$). No differences appeared in the HBR responses acquired in static condition before and after voluntary movement ($p=0.59$).

Further, when we compared HBR responses in Static condition and during voluntary movement a significant interaction between CONDITION and POSITION ($F(4,36)=4.52$, $p=0.005$) was found.

Post doc analysis revealed that AUC values in static condition and in each arm's position were significantly higher than those obtained during voluntary movement, regardless of movement direction (p always < 0.05).

--- Figure 3 here ---

--- Figure 4 here ---

Experiment 2: Passive movement. The statistical analysis showed a significant interaction between POSITION and MOVEMENT DIRECTION ($F(2,18)=6.91$, $p=0.006$). As in the case of voluntary movement, during passive elbow flexion movements (Up-moving condition) the magnitude of the HBR responses significantly increase when the hand was near the face ($\alpha3=11.8\pm1.8$ mV*ms) with respect to far ($\alpha1=6.5\pm0.9$ mV*ms, $p=0.007$) and intermediate ($\alpha2=7.3\pm0.6$ mV*ms, $p=0.02$) positions (Figure 5A), whereas during extension movements (Down-moving condition) no difference was found among the three hand positions ($\alpha1=9.6\pm0.9$ mV*ms, $\alpha2=8.1\pm0.8$ mV*ms, $\alpha3=8.2\pm0.9$ mV*ms, p always > 0.3) (Figure 5B). Finally, the HBR response in $\alpha3$ when the arm was passively moved up-to the face was significantly higher than that observed at the same position when the arm was moved far from the face ($p=0.03$).

RM-ANOVA on the Static condition showed an effect of the factor POSITION ($F(2,18)=8.19$, $p=0.002$), with AUC values in $\alpha3$ (21.66 ± 2.95 mV*ms) significantly higher than those in $\alpha2$ (18.83 ± 2.9 mV*ms, $p=0.006$) and $\alpha1$ (19.14 ± 2.7 mV*ms, $p=0.006$). No differences appeared in the HBR responses acquired in static condition before and after passive movement condition ($p=0.18$).

When we compared HBR responses in Static condition and during Passive movement, a significant interaction between CONDITION and POSITION ($F(4,36)=4.14$, $p=0.007$) was found. Post doc analysis showed that, regardless of movement direction and arm's position, AUC values were significantly higher in Static condition (p always < 0.001).

--- Figure 5 here ---

Experiment 3: Motor imagery (Figure 6). The results of the paired t-test showed a significant effect of the direction of the imagined movement: when participants imagined a flexion movement (Up-moving condition), from the intermediate to the near position, the HBR responses were significantly higher (10.9±0.9 mV*ms) than when they imagined to extend their arm towards the far position (9.9±0.7 mV*ms) ($t=3.04$, $p=0.01$). Further, when these conditions were directly compared to a situation in which the subject kept the arm fixed in α2, the ANOVA showed a significant effect of the factor CONDITION ($F(2,18)=15.09$, $p=0.0001$). The post hoc analysis revealed that MI conditions, irrespective to the imagined movement directions, induced a reduction of the HBR response (p always < 0.0007).

Finally, the statistical analysis on the data acquired in Static condition showed a significant effect of the factor POSITION ($F(2,18)=5.31$, $p=0.01$); AUC values in α3 (17.34±1.34 mV*ms) were significantly higher than those in α2 (14.68±1.3 mV*ms, $p=0.02$) and α1 (15.10±1.1 mV*ms, $p=0.02$). No differences appeared in the HBR responses acquired in Static condition before and the motor imagery task ($p=0.29$).

--- Figure 6 here ---

Discussion

In this study, we sought for evidence that the predictive motor system can modulate a defensive response, the Hand Blink Reflex (HBR). The data discussed here confirm previous evidences on HBR modulation in static condition and provide some important new findings on its modulation in dynamic conditions.

The results of the Preliminary experiment performed in Static condition are in agreement with the previously described “hand position” effect, demonstrating that the HBR is significantly enhanced when one’s own stimulated hand is located inside the DPPS of the face (Sambo et al., 2012a, 2012b; Sambo and Iannetti, 2013).

The Experiment 1 extended beyond the previous research on DPPS passing from static to dynamic conditions. Firstly, we found a significant HBR enhancement in the near position also in dynamic conditions, i.e., when the moving stimulated hand entered inside the DPPS of the face. It is worth noting that, as previously suggested for Static condition (Sambo and Iannetti, 2013), the HBR response was not linearly enhanced through the three positions (far, intermediate and near), but a safety boundary exists: inside this boundary, the stimulus is potentially dangerous; outside, it is safe. Indeed, in both static and dynamic conditions, comparable HBR responses were found when stimuli were received in far (α1) and intermediate (α2) positions. But mostly relevant is the HBR enhancement in the near (α3) position with respect to α1 and α2 only when the hand was moving towards the face and not when the hand was moving down-from the face. In other words, HBR was modulated not only by the position of the hand when receiving the electrical stimulus but also by the direction of the hand movement. This strongly points out that, when the hand is close to the face but the subject is planning to move it far away, the predictive system may anticipate the consequences of the movement. This means that the space representation is dynamically shaped by the movement: the “near” becomes “far”. Thus, what is crucial in HBR modulation it is not the actual position of the stimulated hand, but the future position where the hand is expected to be during the movement. On the other hand, the lack of an increasing of the HBR value when the hand from the far position moves towards the face might suggest that the dynamic shaping of the DPPS interacts with other aspects defining the safety boundary around the body. In particular, the HBR enhancement has a not linear trend: i.e., a significant difference was present only between near position and the other positions. Thus, coherently, the directional modulation could occur only in the near position.

During voluntary arm movements, two sources of information are available to the motor system in order to estimate the final state of the movement: the sensory inflow, like the information coming from vision and proprioception, and the motor outflow (i.e., the copy of the motor commands). Thus, the Experiment 1 results could not disambiguate between the role of these kinds of information in modulating the defensive response. One possibility is that this directional effect only pertains to the voluntary movements, when both the afferent and the efferent information are present. Alternatively, the directional effect could be present also when the afferent and the efferent sources of information are dissociated, as in the passive movements (Experiment 2) and in the motor imagery task (Experiment 3). Our data verified this second hypothesis, showing a directional effect on the HBR modulation both during passive movements and motor imagery task.

Similarly to the voluntary movement, in the Experiment 2, dealing with passive movements, the HBR response increased when the hand received the stimulus near to the face only in the Up-moving condition, while no difference among the three hand positions was found in the Down-moving condition. This means that the modulation of the HBR response occurred also when only the afferent information (coming from vision and proprioception) was available to the system for predicting the consequence of the movement. Van Beers and colleagues (van Beers et al., 1999) showed that a moving hand is localized by a predictive model that describes a multisensory integration as a direction-dependent weighting of the proprioceptive and visual information. In particular, there are at least two different hypotheses about how the weights given to each modality are determined. According to one hypothesis, the weights are determined by the precision of the information in each modality (Pick et al., 1969; Welch et al., 1979); according to second hypothesis, they are related to the attention that is directed to each modality (Canon, 1970, 1971; Uhlarik and Canon, 1971; Kelso et al., 1975; Warren and Schmitt, 1978). In the present study, it would have been also interesting to manipulate the vision versus proprioception in order to investigate the role of these two afferent inputs in modulating HBR separately. However, in a previous study it has been demonstrated that in static

condition the effect of hand position on the HBR persists in absence of vision, thus suggesting a crucial role of proprioception in shaping DPPS (Sambo et al., 2012b).

The results of the motor imagery task (Experiment 3) showed that the HBR response was significantly greater when the subject imagined to move up-to than down-from the face, although the arm was kept in the intermediate position and the position remained unchanged during the experiment. Therefore, whether during voluntary and passive movements no modulation was observed in α2, here the mere movement planning was sufficient to evoke a different HBR response, suggesting the crucial role played by movement intention. Converging evidences suggest that imagined and actual movements trigger similar motor representations (Jeannerod and Decety, 1995; Gentili et al., 2004; Gandrey et al., 2013) and share overlapping neural substrates (Grèzes and Decety, 2001; Jeannerod, 2001; Lotze and Halsband, 2006; Filimon et al., 2007; Hanakawa et al., 2008). In particular, motor imagery would engage the same internal forward models, i.e., the neural mechanisms that mimic the causal flow of the physical process by predicting the future sensorimotor state, that are involved in action execution (Wolpert and Flanagan, 2001). Looking at the present findings, we might propose that the modulation of HBR response obtained during voluntary movements can be fully explained only when considering both the sensory inflow and the motor outflow. Results from the motor imagery task strongly support that the modulation of the defensive response occurred also when no actual movements were executed, but when only the efferent information (i.e., the efference copy of the motor program) was available to predict the final consequences of the movement. These results point out that the cortical brain activations evoked during the motor imagery task affect the brainstem HBR circuits. As suggested by Sambo and colleagues (Sambo et al., 2012b), the HBR enhancement may result from the modulation of the excitability of brainstem circuits mediating the HBR by associative cortical areas in the prefrontal gyrus and in the ventral intraparietal area, involved in the representation of peripersonal space and in the detection of potentially dangerous stimuli near the face. Motor imagery is known to activate a frontoparietal network (Grèzes and Decety, 2001; Bonzano et al., 2016) which includes some of these associative cortical regions identified as top-down modulators of the activity

of the HBR circuitry (Sambo et al., 2012b). Therefore, we might hypothesize that the activation of these areas when the subjects imagine to move the hand in different positions of the DPPS might activate the descending pathways from the associative cortical areas to the medulla in the brainstem and influence the amplitude of the reflex response.

Finally, the higher values of HBR response observed in the Preliminary experiment with respect to the other experiments might suggest the use of two distinct neural mechanisms in modulating HBR when the limb is statically positioned or when the motor system is involved, as in voluntary movement, passive movement and motor imagery. Indeed, for each stimulation position, a significantly reduced HBR was found in dynamic conditions with respect to static conditions. The decrease of HBR response during movement can be explained as consequence of the sensory attenuation, according to which the sensory effects generated by one's own actions are attenuated compared to the same effects generated externally (Blakemore et al., 1999; Tsakiris and Haggard, 2003; Bays et al., 2006; Weiss et al., 2011). It is important to note that, in our experimental context, during voluntary and passive conditions, the participant's movement triggered the electrical stimulator. Thus, the resulting stimulus can be interpreted as a self-generated sensory effect. In the context of DPPS, this means that a stimulus on which I can exert a direct control is interpreted as less dangerous and produces a lower defensive physiological response with respect to an externally generated stimuli, that is, by definition, outside from the subject's control (REF FRA). A similar explanation could account for the results of the motor imagery condition. Indeed, recent studies showed that the sensory attenuation occurs not only during action execution, but also during action preparation and planning (e.g., Lange, 2009). Alternatively, the movement-related HBR decrease can be explained by the different processing of the proprioceptive inputs in static and dynamic conditions. We can suggest that in the static condition the proprioceptive inputs can give an exact information about the position of the hand with respect to the face. Conversely, when a subject is moving, the rapidly changing inputs generated during hand movements can make more difficult to estimate the hand position and therefore might reduce the amplitude of the reflex response. It is worth noting that

455 the movement-related HBR decrease is particularly relevant for the motor imagery condition, where,
456 although the subjects did not actually move, the mere movement planning was sufficient in order to
457 modify the reflex response. Indeed, since motor imagery activates neural circuits partially overlapped
458 to those active during voluntary movement, the mechanisms engaged during the sensory information
459 processing might be similar to those used during the voluntary movement rather than those involved
460 in a static condition. As consequence, the modulation of the HBR response observed during motor
461 imagery might be similar to that activated by an overt movement execution but reduced with respect
462 to the static condition.

463 Taken together, these findings provide physiological evidence for the role of the predictive motor
464 system in dynamically shaping the defensive peripersonal space during movement.

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Figure captions

Figure 1. Experimental set up. Panel A refers to Experiment 1, during which participants voluntarily performed either a flexion-extension or an extension-flexion movement of the elbow: while moving up towards (Up) or down far from (Down) the face they received an electrical stimulation in three pre-set positions (far position, α1; intermediate position, α2; near position, α3). Panel B refers to Experiment 2 and shows the pulley system used by the experimenter to induce the passive flexion-extension (Up) and extension-flexion (Down) movements to participants' right forearm. The electrical stimulation was delivered in the same conditions as in Experiment 1. Panel C refers to Experiment 3 and shows the subject while keeping a static position corresponding to α2 and imagining either an elbow flexion movement towards the face (Up, from α2 to α3) or an elbow extension movement (Down, from α2 to α1).

Figure 2. Preliminary experiment: Static condition. Group-average, rectified HBR waveforms (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) recorded when the arm was placed in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Error bars refer to the standard error of the mean. ** refer to $p<0.01$.

Figure 3. Experiment 1: Voluntary movements. Rectified and superimposed average EMG traces (mean over the blocks and recording sites) of each participant for the near (α1), intermediate (α2) and far (α3) positions when participants performed up movement towards the face (Up-moving) or down movement far from the face (Down-moving).

Figure 4. Experiment 1: Voluntary movements. On the upper (A) and lower (B) panels are represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR

amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Error bars refer to the standard error of the mean. ** refer to $p<0.01$.

Figure 5. Experiment 2: Passive movements. On the upper (A) and lower (B) panels are represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the three stimulation positions: far (α1), intermediate (α2) and near (α3). Error bars refer to the standard error of the mean. * and ** refer to $p<0.05$ and $p<0.01$, respectively.

Figure 6. Experiment 3: Motor imagery. Group-average, rectified HBR waveforms (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) when participants were asked to imagine to move the forearm from the intermediate to the near position (α2 to α3) and from the intermediate to the far position (α2 to α1). Dashed lines indicate the HBR waveform (left panel) and response amplitude (right panel) obtained in static condition corresponding to α2. Error bars refer to the standard error of the mean. ** refer to $p<0.01$.